Seroprevalence of *Mycoplasma gallisepticum* in domestic chickens, East Shewa, Ethiopia

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Abstract

Cross sectional study was conducted to estimate the sero-prevalence of *Mycoplasma gallisepticum*in domestic chickens and to assess risk factors associated with the disease in commercial and local chickens in East Shewa, Ethiopia. A total of 514 sera were collected (from187 commercial chickens and 327 local chickens) and tested using an indirect enzyme linked immunosorbant assay (ELISA) to detect antibodies against *M. gallisepticum*. The overall seroprevalence of *M. gallisepticum* was 49.4% (254/514). A statistically significant association ($p<0.05$) was observed in prevalence between chicken type with prevalence of 64.5% in local and 23% in commercial chickens. Variation in prevalence was observed among the three commercial farms, the highest being in farm-B (46.8%) and the lowest in farm-C (4.25%). Prevalence in local chickens was significantly highest in Lume (72.7%) and lowest in Ada'a (47.5%) ($p<0.05$). Age was significantly associated with sero-prevalence ($p<0.05$). Prevalence was 67.3% in layers of (18 to 76 weeks) and 0% in layer chicks of (1 to 8 weeks old). Prevalence was also significantly different between layers (41.7%) and broilers (7.8%) ($p<0.05$). In commercial chickens, prevalence was significantly higher in females (32.4%) than males (10.1%) ($p<0.05$). The current study revealed *M. gallisepticum* is prevalent in chicken in East Shewa, Ethiopia.

Keywords: Domestic chickens; East Shewa; Ethiopia; *Mycoplasma gallisepticum*; Sero-prevalence

Introduction

Agricultural production dominates the Ethiopian economy and contributes to 45% of gross domestic product (GDP) and provides more than 80% of employment. Ethiopia has the highest livestock populations in Africa and accounts for 17% of cattle, 20% of sheep, 13% of goats and 55% of equines in sub-Saharan Africa. Livestock contributes 16% of GDP. Seventy per cent of cattle, 75% of sheep, 27% of goats and 80% of equines are found in the highlands (Tangka*et al.,* 2002). Of domestic animals in the country, the most numerous are bovines and poultry each estimated 45 million head. This is followed by goats and sheep each at 17 million head, by donkeys (4 million) and by horses and camels (each one million). Poultry are represented exclusively by chickens (FAO, 2008).

In developing countries, poultry production offers an opportunity to feed the fast growing human population and to provide income to the resource poor farmers (Gari, 2004). The sub-sector is concerned with egg and meat production for income generation and home consumption (EARO, 2000; Mohammed, 1998). The main purposes of egg production are hatching (51.8%), sales (22.6%), home consumption (20.2%) and gift (5.4%). The purposes of bird production are sales (26.6%), sacrifice/healing ceremonies (25.0%), replacements (20.3%), home consumption (19.5%) and gift (8.6%) according to Gottard and SoaresMagalhaes (2006).

In Ethiopia, live birds and eggs are usually sold by the owner in local markets. Single bird sales or sales of small numbers typify most rural markets, with many sellers competing. During times of festivity, the numbers and the prices of birds in the market rise considerably, because of demand (Dessie and Ogle, 2001). Occasionally, birds are sold to middlemen for transport and sales in the larger towns and cities. The largest proportion of eggs and poultry meat consumed in the country comes from indigenous birds produced by rural growers. Large numbers of these birds are also exported to neighboring countries within trade that is mainly informal. Therefore, the main movement of poultry and poultry products is one of rural producer to urban consumer and from Ethiopia to neighboring countries, which from an Ethiopian biosecurity point of view is profitable, because it is not favorable to the diffusion of poultry diseases all over the country (FAO, 2008).

Mycoplasma gallisepticum (MG), the etiologic agent of chronic respiratory disease (CRD), is one of the major pathogens of domestic poultry causing signifi-

cant economic losses particularly to the commercial poultry industry resulting in reduced feed conversion, reduced egg production and significant downgrading of carcasses at slaughter. The disease has a worldwide distribution and is extremely important to both the broiler grower and the table egg producers. Infection of air sacs in broilers (air sacculitis) is the cause for condemnation of dressed birds as unsuitable for human consumption. Laying flocks positive for MG have been shown to produce as many as 20 fewer eggs per year than MG negative flocks (Garcia *et al*., 2001; Talha, 2003).

With the huge population of chickens being reared and the industry steadily growing, major disease problems of commercial and local chickens have not been well investigated in Ethiopia. There is no documented work on the status and distribution of MG infection in Ethiopia. The objectives of this study, therefore, were to estimate the sero-prevalence of *M. gallisepticum* in commercial and local chicken production systems in East Shewa, Ethiopia, and to assess risk factors for the sero-prevalence.

Materials and methods

Study area

The study area, East Shewais located in the center of Ethiopia with area of 14,050.27km^2. Ithas an altitude range of 3100 to less than 1000 meter above sea level (masl). It is located in the middle of Oromia, connecting the western regions to the eastern ones. This zone is bordered on the south by the West Arsi Zone, on the southwest by the Southern Nations, Nationalities and Peoples Region, on the west by South west Shewa and Oromia Special Zone Surrounding Addis Ababa, on the northwest by North Shewa, on the north by the Amhara Region, on the northeast by the Afar Region, and on the southeast by Arsi; its westernmost reach is defined by the course of the Bilate River. Towns and cities in East Shewa include Bishoftu (DebreZeit), Metehara, and Ziway. The town of Adama was separated from East Shewa and is a special zone now. Five woredas were randomly selected: Ada'a, Boset, Gimbichu, Lume, and Adami Tulu. Gimbichu lies in the highland with about 2450 masl,Ada'a and Lume fall under mid-high land of 1600-2300 masl, whereas Boset and Adamitulufall under low land with an altitude range of 1200-1500 masl (https://wikipedia.org/wiki/EastShewaZone, Web retrieved May, 2018).

Study animals

A total of 514 chickens sera were collected (187 from commercial and 327 from local chickens). For local chicken production, chickens were randomly bought from market places of the selected five Woredas, whereas, commercial chickens (layers and broilers) were obtained from the three selected commercial poultry farms in Bishoftu, East shewa, Ethiopia, which were coded, for confidentiality, as farm-A, farm-B and farm-C. The farms undertake regular vaccination against New Castle Disease (NCD), Gumboro (IBD), fowl typhoid, fowl pox, and Infectious Bronchitis (IB). The housing was deep litter system for broilers and layer chicks: whereas, battery cage system for layers. Feeding and watering system was through automatic feeders and waterer. Birds were supplemented with minerals, amino acids, and vitamins produced locally and imported. The layer breeds were of Bovan-Brown, Rod Island Red (RIR), and Lohmann; and broiler breeds were of Cobb-500 and Rose. Layer and broiler flocks of different age were included (Table 1).

Table 1. Study chickens with different age group, sex, and number of samples taken

Age group and bird type	Production system	
	Commercial chickens	Local chickens
Broiler		
Grower (3 to 6 weeks)	61	-
Finisher (6 to 10 weeks)	42	-
Layer		
Chicks (1 to 8 weeks)	32	-
Layers (18 to 76 weeks)	52	-
Local		
Adult local	-	327
Sex		
Male	79	145
Female	108	182
Total	187	327

In farm-A, both layers and broilers with one flock of layer chicks and one flock of commercial layers, and two flocks of broiler growers and two flocks of broiler finishers were considered; in farm-B, layer flocks with one flock of layer chicks and one flock of commercial layers; and in farm-C, broiler flocks with one flock of broiler finisher and two flocks of broiler growers were considered for the

study (Table 2). None of the commercial chickens were vaccinated against MG infection.

Table 2. Number of commercial chickens (layers and broilers) sampled

Commercial chickens	Farm-A	Farm-B	Farm-C	Total
Layers				
Chicks	19	13	-	32
Layers	18	34	-	52
Broilers				
Grower	40	-	21	61
Finisher	16	-	26	42
Total	93	47	47	187

Study design and sample size determination

Cross sectional study with stratified random sampling was followed. Poultry production system was stratified into commercial and local chicken production systems. In commercial, layer flock chicks (1 to 8 weeks old) and layers (18 to 76 weeks), and broiler flocks with broiler grower (3 to 6 weeks) and finisher flock (6 to 10 weeks) were included for the study. Chickens of both sexes and different age groups were stratified and sampled randomly. In the local chicken production system, an average adult chicken of both sexes were bought from different market places from the selected five Woredas of East Shewa. The effects of sex, age, bird type (layer, broiler), and production system were assessed on the sero-prevalence of *M. gallisepticum* infection. Sample size was determined using Win-episcope 2.0 (Thrusfield *et al*., 2001). The sample size thus, determined was 385; however, maximized to 514 to increase precision. Proportional sampling was followed assuming the dominant system of chicken production in the study area is local chickens and hence, a total of 187 sera from commercial chickens and 327 sera from local chicken were collected.

Sample collection

About 3 to 4 ml of blood sample was collected from the wing vein using 5 ml sterile syringe and needle, and kept at room temperature for 6 to 8 hours until the blood clots. Then, the serum was harvested into sterile cryo-tubes, recorded individuallyand stored at -20^{o}C until processed at the National Veterinary Institute (NVI), Bishoftu, Ethiopia.

Laboratory methodology

Indirect enzyme linked immune sorbent assay (ELISA) was employed to detect antibodies against MG infection. The antibody ELISA test kit (FLOCK SCREEN Cat. No. V050/V054) GUILDHAY Company, Surrey, England was used. The test procedure was followed according to the manufacturer's instruction. Unknown test samples were tested in parallel with positive and negative controls.

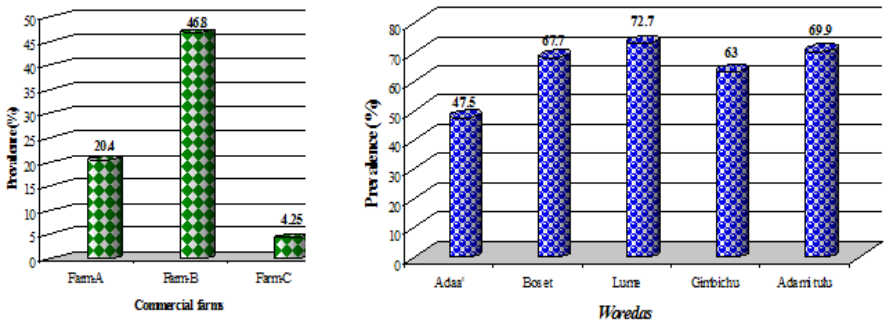
Data analysis

Data entry was made on MS-excel and analyzed using statistical package-STATA version-intercooled stata 7.0. Summary statistics were used to estimate sero-prevalence of *M. gallisepticum*. Pearson chi-square and univariate logistic regression were used to assess association between risk factors of production system, age, bird type, and sex on the prevalence of MG infection; and significant association at a 95% confidence level and p-value of 5% as statistical significance.

Results

The overall sero-prevalence of *M. gallisepticum* was 49.4% (254/514). The prevalence for local chickens and commercial chickens were 64.5% and 23%, respectively, and the difference was found to be statistically significant ($p<0.05$). The sero-prevalence of MG infection was found to be significantly associated with poultry production systems ($p<0.05$, χ^2=82.09, OR=6.09). Risk ratio of 2.8, risk difference of 41.5%, attributable fraction among local chickens of 64.4%, and attributable fraction among population of 53.5%, were recorded.

Of the three commercial poultry farms, prevalence was high in farm-B (46.8%) as compared to the lowest value observed in farm-C (4.25%); this difference was statistically significant ($p<0.05$). Moreover, of the five Woredas, the highest prevalence was observed in Lume (72.7%) and the lowest in Ada'a (47.5%) and this was statistically significant ($p<0.05$) (Figure 1 and Table 3).



(A) (B)

Figure 1: Sero-prevalence of MG in commercial chicken farms (A) and local chickens of the Woredas **(B)**

Table 3. Sero-prevalence of MG with confidence intervals of the farms and Woredas

No	Farm/Woreda	No. sampled	No. positive	Prevalence (%)	95% CI
1	Farm-A	93	19	20.4%	12.1% - 28.8%
2	Farm-B	47	22	46.8%	32% - 61.6%
3	Farm-C	47	2	4.25%	0% - 10.2%
4	Ada'a	61	29	47.5%	34.6% - 60.4%
5	Boset	93	63	67.7%	58.1% - 77.4%
6	Lume	44	32	72.7%	59% - 86.4%
7	Gimbichu	46	29	63%	48.6% - 77.5%
8	Adamitulu	83	58	69.9%	60% - 80%
	Overall	514	254	49.4%	45.1% - 53.7%

Among commercial chickens, significant difference ($p<0.05$) in prevalence was demonstrated between layers (41.7%) and broilers (7.8%). Age was significantly associated ($p<0.05$, χ^2=55.3, OR=3.8) with MG sero-prevalence and overall increase in prevalence with increasing age was recorded. In layer flocks, there were no seropositives in chicks between 1 to 8 weeks; however, a prevalence of 67.3% was recorded in layers between 18 to 76 weeks and this was statistically significant ($p<0.05$). In broiler flocks, prevalence of 6.6% in broiler growers (3 to 6 weeks) and 9.5% in finisher broilers (6 to 10 weeks) was recorded, however, this was not statistically significant ($p>0.05$) .

Sex was significantly associated ($p<0.05$, χ^2 =5.11, OR=1.497) with MG sero-prevalence among study chickens with higher prevalence in females (53.8%) than males (43.8%). There was no statistically significant association ($p>0.05$) between sero-prevalence of MG i and sex of local chickens investigated although it was slightly higher in females (66.5%) than males (62.1%). However, statistically significant association ($p<0.05$) was observed between sero-prevalence of MG and sex of commercial chickens with 32.5% in females and 10.7% in males.Poultry production system, bird type (broiler and layer)and age were significantly associated with MG sero-prevalence (Table 4).

Table 4. Summary of OR, 95% CI of OR, and p-values of epidemiological risk factors associated with MG infection using logistic regression

Risk factors	OR*	95% CI of OR	SE* of OR	Chi^2 (LR)*	P-value
Production system	6.091	4.046 - 9.17	1.27	85.5	0.000
Age	2.51	2.05 - 3.08	0.26	135.62	0.000
Bird type (breed)	8.48	3.655 - 19.68	3.64	31.3	0.000
Sex	1.497	1.05 - 2.126	0.27	5.11	0.024

OR*= Odds ratio, SE*= Standard error, Chi^2(LR)* = Chi square of logistic regression

Discussion

Previous investigations showed that MG infection represents a major problem of chickens reared in commercial poultry farms (Pradhan *et al.,* 2000; Salequ*et al.,* 2003). The study of Talha (2003) demonstrated that MG infections are not only widespread in commercial layer and broiler chickens but also in local (village, backyard) chickens. Similarly, the present study revealed that MG is prevalent in both commercial and local chickens. Although much work has been done on the prevalence of MG infection in chickens in many countries of the world, there are wide variations in the seroprevalence of MG reported due to differences in selecting target population, sample size estimation and tests employed (Talha, 2003). The overall sero-prevalence of MG in the present study (49.4%) was in agreement with the studies of Abdu *et al* (1983) in Nigeria and Sikder *et al* (2005) in Bangladesh who reported seroprevalence of 47.5% and 46.9%, respectively. However, the present finding was lower than the investigation of Sarkar *et al* (2005) who reported a prevalence of 58.9% in model

breeder poultry farms of Bangladesh. The higher sero prevalence recorded in the local chickens might be attributed to scavenging type of feeding, associated nutritional deficiencies, and lack of disease control program in the local poultry production system which contributed to the increased risk of exposure to MG. Moreover, local chickens can be gathered at different market places which increase the risk of exposure. The local chickens were apparently healthy and could serve as a source of infection for commercial chickens. On the other hand, sero-prevalence was lower in commercial chicken breeds because of better hygienic and management practices, and better disease control program as intensive production system.

The prevalence in commercial chickens (23%) in the present study was higher when compared to the report of Mohammed *et al* (1986) in central California in commercial pullets and layers (3%) and of Zelenika*et al* (1999) in Croatia in heavy hen hybrids in 1997 (10%) and in 1998 (13%). On the other hand, the current finding was lower when compared to the findings of Mohammed *et al.,* (1986) in commercial pullets and layers in southern California (73%), Zhang *et al* (2001) on broiler chickens in Mongolia (53%), and Godoy *et al* (2001) on layer chickens in Venezuela (66%). The present finding was in agreement with the report of Godoy *et al* (2001) on layer chickens in Venezuela which was 22%. The prevalence of local chickens (64.5%) in the present study was higher than the study by Kelly *et al* (1994) in Zimbabwe with a prevalence of 33%, and that of Shah-Majid (1996) in Malaysia with 26% prevalence. However, the current finding of MG sero-prevalence in local chickens was in close agreement with the study of Chrysostome *et al* (1995) in local chickens in Benin with a prevalence of 62%.

Among commercial poultry farms, the highest prevalence was recorded in farm-B (46.8%) and the lowest in farm-C (4.3%). The highest prevalence recorded in farm-B might be due to being a continuous (multi-age) production site where different age groups of layer flocks were kept in the same farm. Butcher (2002); Talha (2003) and OIE (2004) indicated that MG infection is likely to occur in multi-age production sites, which is common in layer complex and multi age breeder sites. In farm-A, different age groups of layer flocks were kept in houses far apart relative to farm-B. Farms A and C have adapted all-in-all-out principle where the possibility of disease introduction and spread is relatively minimum. Variations observed in prevalence between the farms might also be due to some faulty management and bio-security practices.

In local poultry production, the highest prevalence was recorded in Lume Woreda (72.7%) and the lowest in Ada'a (47.5%). Sarkar *et al* (2005) demonstrated significant variation in the prevalence of MG infection between districts in Bangladesh. However, the study by Talha (2003) showed no significant difference in prevalence among local chicken flocks investigated at different districts in Bangladesh. Lume Woreda is located on the main road connecting eastern and southern parts of the country to the capital where movement of people and livestock including chickens is high and probably contributing to the highest prevalence of MG recorded in the Woreda.

Age was significantly associated with sero-prevalence of MG where an increasing prevalence with an increasing age was observed. Age variation in sero-prevalence of MG was reported by many authors: Talha (2003) in local (backyard) chickens in Bangladesh reported increasing prevalence with increasing age and also showed that older birds were more seropositive to MG than younger birds, which agrees with the present finding. However, Sikder *et al* (2005) and Sarkar *et al* (2005) in model breeder poultry farms in Bangladesh reported a decreasing prevalence with an increasing age which contradicts with the current finding. Association of age with prevalence of MG might be due to an increase in disease risk with an increasing age particularly in commercial production where birds enter into different production stresses as they get older. In the local poultry production system, only the same average adult local chickens were considered and age variation in prevalence was not appreciated.

The prevalence in layer chickens in this study (41.7%) was lower than the report of Godoy *et al* (2001) in layer chickens in Venezuela, and in commercial pullets and layers in southern California (Mohammed *et al.,* 1986); but higher than the finding of Godoy *et al* (2001) and Mohammed *et al* (1986) who reported 22% and 3%, respectively. The prevalence record of broilers in the present finding (7.8%) was lower than the record of Zhang *et al* (2001) who reported 53% prevalence and close to the finding of Zelenika *et al* (1999) in heavy hybrids in Croatia with 10% in 1997 and 13% in 1998. The risk of infection in layers was high which might be due to the stress associated with egg production and the multi age production associated in layer flocks where infection is more likely to occur than the all-in-all-out system of production in broiler flocks. The slightly higher prevalence recorded in females in local chickens might be due to the large number of females sampled relative to males. However, significant association between sex and prevalence of MG infection was demonstrated among commercial chickens being higher in females (32.5%) than males (10.7%). This

might be due to the fact that female birds are under high stress of egg production. In addition, in multi age production units where mostly female chickens are kept and infection is likely to occur, especially when layer pullets get into the laying complex, might also contribute to the high prevalence recorded in females. This finding agrees with the work of Sarkar *et al* (2005) and Sikder *et al* (2005) who reported higher prevalence of MG infection in females than males with prevalence of 59.9% and 24.1% in females, and 48.6% and 15.6% in males, respectively.

Conclusion

This study revealed that *Mycoplasma gallisepticum* (MG) infection is prevalent and widespread in both commercial and localchickens. The highest prevalence in local chickens demonstrated the risk to the huge population of local chickens and might also serve as a source of infection for commercial chickens. Thesero-prevalence finding in the present study indicated that the organism is probably circulating among the population of chickens; and it was concluded that this study should be substantiated by isolation and molecular characterization of *Mycoplasma gallisepticum* in domestic local chickens.

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Conflict of interest

The authors declare that there is no conflict of interest.

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